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DOI: https://doi.org/10.1016/j.aquatox.2014.01.018

Molecular effects of the cyanobacterial toxin cyanopeptolin (CP1020) occurring in algal blooms: global transcriptome analysis in zebrafish embryos

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Abstract

Higher water temperatures due to climate change combined with eutrophication of inland waters promote cyanobacterial blooms. Some of the cyanobacteria produce toxins leading to drinking water contamination and fish poisoning on a global scale. Here, we focused on the molecular effects of the cyanobacterial oligopeptide cyanopeptolin CP1020, produced by *Microcystis and Plankthothrix* strains, by means of whole-genome transcriptomics. Exposure of 72 hpf old zebrafish embryos for 96 h to 100 and 1000 µg/L CP1020 resulted in differential transcriptional alteration of 396 and 490 transcripts (fold change ≥2), respectively, of which, 68 gene transcripts were in common. These belong to genes related to various important biological and physiological pathways. Most clearly affected were pathways related to DNA damage recognition and repair, circadian rhythm and response to light. Validation by RT-qPCR showed dose-dependent transcriptional alterations of genes belonging to DNA damage and repair and regulation of circadian rhythm. This leads to the hypothesis that CP1020 acts on DNA and has a neurotoxic activity. This transcriptome analysis leads to the identification of novel and unknown molecular effects of this cyanobacterial toxin, including neurotoxicity, which may have important consequences for humans consuming contaminated drinking water.

Key Words: Cyanobacterial toxin, cyanopeptolin, transcriptomics, modes of action, molecular effects, zebrafish

1. Introduction

Cyanobacteria can form dense blooms in many aquatic systems, one of the most bloom triggering factors being the nutrient load. Due to eutrophication, an increased frequency of cyanobacterial blooms may be observed worldwide (Carmichael, 2008). Furthermore, this increase can be related to global warming (Paerl and Paul, 2012; Posch *et al.*, 2012). Thick mats of cyanobacteria deteriorate recreational areas, can block the sunlight from reaching deeper water layers and cause an anoxic environment during decomposition.

Cyanobacterial blooms raise concerns about their ecotoxicological and human health implications due to the formation of bioactive secondary metabolites. Their presence is frequently observed in different cyanobacterial genera such as *Microcystis*, *Anabaena*, and *Planktothrix*. Some of the most toxic natural compounds are found among cyanobacterial toxins, including the highly hepatotoxic microcystins (MC). Microcystins are one of the most abundant cyanobacterial toxins, mainly produced by *Microcystis* and *Plankthotrix* species (Sivonen and Jones, 1999). During decomposition of toxic and so called "harmful algal bloom", these toxins contaminate waters, causing illness or death of other organisms including humans (Azevedo *et al.*, 2003; Poste *et al.*, 2011). MCs are cyclic heptapeptides that inhibit serine/threonine-specific protein phosphatases 1 and 2A (McKintosh *et al.*, 1990), and that bind to the beta chain of the ATP-synthase unit (Mikhailov *et al.*, 2003). Moreover, MCs lead to endoplasmatic reticulum stress (Christen *et al.*, 2013) and, due to their tumor promoting activity, they are considered to be responsible for a higher rate of liver cancer in China (Ueno *et al.*, 1996) and Serbia (Svirčev *et al.*, 2009). For MC-LR, the most abundant of more than 90 different MC congeners, the WHO set a provisional guideline in drinking water of 1 µg/L (Burch 2008).

Due to its high toxicity, research has mainly focused on MC, whereas other cyanobacterial toxins have only received little attention. However, there is a great diversity of cyanobacterial secondary metabolites, including oligopeptides, but their potential adverse effects to organisms remain largely unknown. Cyanopeptolins (CP) are peptides widely distributed amongst cyanobacteria, and mainly known for their inhibition of serine proteases like chymotrypsin or trypsin (Blom *et al.*, 2006; Bister *et al.*, 2004). CP1020 was isolated from *Microcystis* and its acute toxicity (LC_{50} = 8.8 µM) to the crustacean *T. platyurus* was comparable to that of microcystins (Blom and Jüttner, 2005). Moreover,

CP1020 showed very potent inhibitory activity to crustacean and mammalian serine proteases (Gademann *et al.*, 2010)**.** Similar to MC, CP1020 is assumed to enter fish cells by active transport processes. However, so far nothing is known about its uptake mechanism, molecular effects and modes of action to fish and mammals.

In general, the biological functions and toxic action of cyanobacterial oligopeptides are not well understood. Effects of natural and anthropogenic compounds on the gene expression level can occur even before physiological consequences can be observed (Christen *et al.*, 2011; Oggier *et al.*, 2010; Yang *et al.*, 2007; Zucchi *et al.*, 2011). Therefore, investigations of transcriptional alterations can provide insights into molecular mechanisms underlying a toxic response. In addition, transcriptomal effects in zebrafish may be a surrogate for effects in mammals. Using global transcription analysis (transcriptomics), expression pattern of thousands of genes can be analyzed simultaneously, allowing a detailed comparison between organisms exposed to a toxin and control organisms (Fent and Sumpter, 2011). Recently, molecular effects of MC-LR on zebrafish embryos have been analyzed by transcriptome analysis (Rogers *et al.*, 2011), the only cyanobacterial toxin investigated by this advanced method in fish so far. The aim of the present work was to evaluate the molecular effects and mode of actions of the novel cyanobacterial toxin CP1020 in zebrafish eleuthero-embryos by means of microarrays. The obtained data shed new lights on the potential ecotoxicological and human health risks originating from toxins from cyanobacterial blooms, which are of increasing concern due to eutrophication and global warming.

2. Material and Methods

2.1 Culture conditions of cyanobacteria. *Microcystis aeruginosa* strain UV006 was cultured in 300 mL Erlenmeyer flasks at 20 °C under constant light conditions at an irradiation of 6 µmol / m^2 s from fluorescent tubes (Osram 930; Lumilux Delux; Warm White 3000K) in 120 mL mineral medium (Jüttner *et al.,*1983).

105 **2.2 Separation and mass spectrometrical analysis of the oligopeptides.** Frozen biomass was
106 extracted twice with 60% MeOH (10 mL per g wet weight) for 2 h in the dark. After centrifugation
107 (25'700 g for 15 min) the supernatant (crude extract) was separated by HPLC equipped with a
108 photodiode array detector using a reversed phase column (Hydrosphere C18, YMC, 4.6 x 250 mm,
109 Stagroma, Reinach, CH; ODS-A, 4.6 x 250 mm, Stagroma, Reinach, CH) under the following
110 conditions: solvent A was UV-treated deionised water (+0.05% trifluoroacetic acid; TFA), solvent B:
111 HPLC-grade acetonitrile (+0.05% TFA). A linear increase in three steps was applied (solvent B: from
112 30% to 35% in 10 min, from 35% to 70% in 30 min, 70 to 100% in 2 min, isocratic for additional 10
113 min). Mass spectra were recorded on a combined LC-MS (LCQ Duo mass spectrometer, Finningan
114 Thermoquest, USA) equipped with an electrospray ionization source (ESI-MS). Cyanopeptolin 1020
115 was collected by HPLC and purified to eliminate TFA as it may lead to undesirable isomerization
116 products of some oligopeptides by using C18 cartridges (1 g, 60 mL, Mega Bond Elute, Varian,
117 Agilent Technologies, Basel, CH) and MeOH (ROTISOLV ≥99.95%, LC-MS-Grade, Roth, CH).
118 Chromatograms of absorption and masses of the detected CP1020 are shown in the supplementary
119 data (Figure S1). Purity was found > 98% by absorption.

120

121 **2.3 CP1020 preparation**. A 10 mM stock solution was prepared from the lyophilized CP1020 in
122 dimethyl sulfoxide (DMSO; from Sigma Aldrich, Fluka AG, Buchs, Switzerland) and further diluted
123 to two stock solutions of 1 g/L and 10 g/L CP1020. The latter was then diluted with reconstituted fish
124 water to obtain final nominal concentrations of 100 µg/L and 1000 µg/L CP1020 (0.01 % DMSO).

125

126 **2.4 Eleuthero embryos exposure**. Fertilized eggs were obtained from Harlan Laboratories Ldt.
127 (Itingen, Switzerland). After quality control under the stereo-microscope (Zeiss, D4), early embryos
128 were transferred to 150 mL glass beakers (80 embryos per beaker, 16 beakers in total) containing 100
129 mL of freshly prepared reconstituted fish water (deionized water with ions added: 61.6 mg/L $MgSO_4$
130 x 7 H_2O; 147 mg/L $CaCl_2$ x $2H_2O$; 32, 4 mg/L $NaHCO_3$ and 2.9 mg/L KCl and a conductivity of 470 –
131 480 µS/cm). Beakers were covered with Petri dishes and held in an incubator with temperature set to
132 27 ± 1 °C and a photoperiod of 16:8h light/dark for development of zebrafish embryos.

Similarly to Rogers *et al*., 2011, static exposure started at 72 h post fertilization (hpf). A total of 60 hatched eleuthero-embryos out of the 80 embryos from each beaker were transferred into a new autoclaved beaker containing 100 mL of freshly prepared media with the appropriate concentrations of CP1020. Exposure to CP1020 was performed in four dose groups, water control, DMSO solvent control (0.01 % DMSO), 100 and 1000 µg/L CP1020. For each dose groups, 4 replicates (independent biological replicates) at each nominal concentration of 100 and 1000 µg/L and 4 replicates of water control and solvent control (0.01% DMSO) were included.

Limited quantity of purified Cyanopeptolin for eleuthero-embryo exposure reduced the overall amount of exposure water collected for chemical analysis and the total number of water exchanges performed during the exposure period. In fact, exposure water (100 mL) were taken at the beginning (0 h) from freshly prepared dilutions and after 4 days (96 h) of exposure, directly from each of the four replicates. Samples were stored at -20 °C until further analysis by HPLC.

Every 24 h, normal development and viability was controlled under the stereo-microscope. Zebrafish eleuthero-embryos were sacrificed after 96 h (168 hpf) for RNA extraction and subsequent microarray analysis.

2.5 Total RNA extraction, microarray hybridization, and sample selection. Eleuthero-embryos from each replicate (n=4) were randomly separated into two groups of 30 individuals, pooled in RNA-Later and stored at -80°C. Total RNA was extracted from zebrafish pools (*n*=30) using the RNeasy Mini Kit (Qiagen, Basel, Switzerland). RNA concentrations and RNA purity were measured spectrophotometrically using a NanoDrop ND-1000 UV-VIS spectrophotometer, and RNA integrity was controlled using the Biorad Experion automated electrophorese system, and an Agilent 2100 Bioanalyzer (Agilent Technologies, Basel, Switzerland). Only samples with a 260/280 nm ratio between 1.8 – 2.1, and an RNA integrity number (RIN) > 8 were used for the hybridization. RNA processing and hybridization for transcriptome analysis was performed by the Functional Genomics Centre (FGCZ), ETHZ and University of Zürich. Global transcriptome analysis was performed as previously described (Oggier *et al.*, 2010; Zucchi *et al.*, 2011).

For transcriptomic analysis, two CP1020 concentrations and the solvent control were used. Transcriptional changes induced by CP1020 were determined by comparison of CP1020-treated

161 embryos to those of the solvent control treated with the identical amount of solvent (0.01% DMSO). A
162 total of 12 arrays (Agilent 4 x 44 K Zebrafish microarray, each array contains barely 43803 probes),
163 were used, array was used for each replicate and three independent arrays were used for each
164 treatment. 600 ng of total RNA were reverse-transcribed into double strand cDNA in the presence of
165 RNA poly-A controls with the Agilent One Color RNA Spike-In Kit. Cy3 labeling and hybridization
166 were performed by the Functional Genomics Centre (FGCZ), ETH and University of Zürich,
167 according to the manufacturer's manual.
168
169 **2.6 RT-qPCR analysis.** Validation of microarray results was performed by real-time quantitative
170 reverse transcription polymerase chain reaction (RT-qPCR) analysis of selected target genes including
171 nuclear receptor subfamily 1 (*nr1d1*), period homolog 1a (*per1a*), cryptochrome 5 (*cry5*),
172 prostaglandin D2 synthase (*ptgds*), vitellogenin type 2 (*vtgII*), estrogen receptor alpha (*esr1*), estrogen
173 receptor beta 1 (*esr2b*), aryl hydrocarbon receptor nuclear translocator 2 (*arnt2*), *Danio rerio* ATP-
174 binding cassette, sub-family G, member 2a (*abcg2a*), and sub-family C (CFTR/MRP), member 2
175 (*abcc2*) (Table S1). The ribosomal protein RPL was used as internal standard (housekeeping gene).
176 Gene specific primers were obtained from published sequences or designed using NCBI Primer Blast
177 and listed in Table S1.

178 First, 1 µg RNA was reverse-transcribed by Moloney murine leukemia virus reverse transcriptase
179 (Promega Biosciences, Inc., Wallisellen, Switzerland) in the presence of random hexamers (Roche)
180 and deoxynucleoside triphosphate. Subsequently the reaction mixture was incubated for 5 min at 70
181 °C and then for 1 h at 37 °C, and at the end for 5 min to 95°C to stop the reaction. The mRNA quantity
182 was then determined using SYBR green (SYBR green PCR master mix; Roche) in a Biorad CFX 96
183 Real Time System. Amplification conditions were 95 °C for 5 min, 40 cycles of 95°C for 30 s, and 57-
184 60^{o}C at primer specific annealing temperatures (*cry5* and *nr1d1* 62.5°C; *per1a*, *esr2b*, *arnt2*, *abcc2*
185 and *vtgII* 60.7 °C; *ptgds* and *esr1* 58.6 °C) for 60 s. A melting curve post run(65-95 ^{o}C) was
186 performedto confirm the specificity of the chosen primers as well as the absence of primer dimers. In
187 addition correct PCR product sizes were checked by an agarose gel. Each reaction was run at least in
188 duplicate.

Efficiency of the PCR reactions was determined by generating a standard curve. Ct values resulting from a reaction mixture with template diluted 1:10 in four steps were plotted against the log of the starting quantity. Expression levels of selected genes were calculated using the $2^{\Delta\Delta Ct}$ method. All gene expression data are reported as log2 transformed.

2.7 Data analysis and statistics. The raw microarray data obtained from the FGCZ were processed according to Oggier *et al.,* 2010 and Zucchi *et al*., 2011, using GeneSpring GX 11.5 software (Agilent Technologies). Here we show the data only for strong alteration with a minimal two-fold difference compared to the solvent control (FC ≥2) (supplementary information). In a first step, the Agilent Feature extraction software output was filtered on the basis of feature saturation, non-uniformity, pixel population consistency, and signal strength relative to the background level (Agilent Feature Extraction Manual). Only positively marked entities, in which at least 50% of the values for two out of three conditions, were accepted for further evaluation. All data were quantile normalized. In a second step, several quality control steps (correlation plots and correlation coefficients) using the quality control tool of GeneSpring were performed to ensure that the data were of good quality. In addition, a quality report was provided by the FGCZ, sample clustering is shown in Figure 2.

Differentially expressed genes from the microarray were determined using a Benjamini-Hochberg multiple correction-ANOVA test ($p < 0.05$ and the fold change (FC absolute ≥2). To determine gene ontology (GO) categories of differentially expressed genes, the GO analysis tool in GeneGo (GeneGo, San Diego, CA, Version 6.3, http://www.genego.com) was used. Enrichment was examined in all three major GO categories (e.g., biological process, cellular component, molecular function), but only biological process results are reported here, as they were the most relevant category for the purposes of this study. Only those categories where $p < 0.05$ are considered differentially altered. MetaCoreTM (GeneGo, San Diego, CA, Version 6.3) from GeneGo Inc. (http://www. genego.com) was used to identify and to visualize the involvement of the differentially expressed genes in specific pathways (FDR< 0.05). Data from qRT-PCR were illustrated graphically with GraphPad_Prism 5 (GraphPad Software, San Diego, CA, USA). Differences between treatments were assessed by one-way ANOVA followed by a Tukey test (Bartelett test $p > 0.05$) to compare means of treatments with (solvent-)

controls. Results are given as means ± standard error of means. All transcriptomics data are reported as log2 transformed here.

Results

3.1 CP1020 concentrations and gross toxicological parameters. The concentrations of CP1020 in exposure waters were close to nominal. At the beginning 128 µg/L and 1185 µg/L CP1020, respectively, were determined in the low and the high dose groups (Table 1). After 96 h the CP1020 concentration decreased to approximately 70 % to average concentrations of 90 µg/L and 856 µg/L, respectively. As average concentrations during the 24 h static-renewal exposure were close to nominal, results are presented here as nominal concentrations. No CP1020 was detected in control groups. No mortality or abnormal behavior of zebrafish embryos was recorded during exposure to these concentrations.

3.2 Differential gene expression in CP1020 exposed zebrafish eleuthero-embryos. Exposure to the low and high CP1020 concentrations resulted in differential expression of 390 and 490 genes (fold change ≥ 2, $p \leq 0.05$), respectively as illustrated in the Venn Diagram (Figure 1) and listed in the Supplementary Material (Table S2, Table S3, Table S4).

The total set of raw data has been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE50139 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE50139).

Sample clustering analysis (Figure 2) (performed by the Functional Genomic Center Zurich) clearly revealed partitions among control and treated samples.

Since all hybridizations met the quality requirements, no data were excluded from further analysis. Cluster analysis is given in Figure 2. Exposure to 100 µg/L CP1020 resulted in 396 significantly (≥ 2 fold change absolute, $p \leq 0.05$) altered transcripts (328 transcripts unique to this dose), and exposure to 1000 µg/L in 490 altered transcripts (422 unique to this dose). A total of 68 genes differentially

regulated were common to both treatment groups (Table S2), with 20 (29.4 %) up-regulated and 48 (69.1 %) down-regulated. One gene, *glrx5* (glutaredoxin 5 homolog 1a), was complementary regulated in the dose groups. At 100 µg/L the majority of genes were up-regulated (82.1 %), while the majority of genes were down-regulated at 1000 µg/L (59.2 %).

Pathway analysis of differentially expressed genes was performed by MetaCore TM software. At both CP1020 concentrations, the top 10 scored pathway maps (revealed by comparison workflow analysis) are listed in Table 2a. Altered transcripts belong to genes and pathways involved in DNA damage and repair, p53 signaling and the neurophysiological process circadian rhythm among others (including heme metabolism). GO processes of genes that respond to CP1020 treatment were also analyzed by MetaCore software and the top ten processes are listed in Table 2b. Strongly regulated are genes belonging to the processes nucleotide-excision repair and DNA damage recognition, catabolic and biosynthetic processes. The lists of altered transcripts unique to 100 and 1000 µg/L, respectively, are given in Tables S3 and S4.

3.4 Validation of microarray data by quantitative real time PCR of selected genes. Based on MetaCore analysis, genes belonging to the top scored pathways and some additional genes were selected for validation of the array data. Additionally, dose-response relationships of selected genes are evaluated. The obtained microarray and quantitative RT-qPCR data are depicted in Figure 3 and Figure S2.. Nuclear receptor subfamily 1, group D member 1 (*nr1d1*), and the period homolog 1a (*per1a*), both involved in circadian rhythm (Vatine *et al.*, 2011), are significantly down-regulated in both dose groups. This is reflected in the microarray data, and confirmed by RT-qPCR. Cryptochrome 5 (*cry5*) is related to circadian rhythm (Cashmore *et al.*, 1999), but functions in DNA damage repair (Hirayama *et al*., 2009). Down-regulation of *cry5* is demonstrated both by means of microarray and RT-qPCR analysis, showing a good correlation between the two methods. Furthermore, prostaglandin D2 syntethase D2 (*ptgds*) is down-regulated, as shown by microarrays and validated by RT-qPCR. Prostaglandin D2 is known to mediate sleep, body temperature and hormone release (Mong *et al.*, 2011).

The microarray data also show transcriptional alterations of estrogen receptors *esr1* and *esr2b*, which was also analyzed by RT-qPCR (Figure S2). In addition, two ABC-transporters, *abcc2* and *abcg2a*, and *arnt2* are analyzed by RT-qPCR (Figure 3, Figure S2), but the alteration found by microarray lack confirmation for *abcc2 and arnt2*, could only partly confirmed for *abcg2a,*and *vtg2* could not be amplified. Thus, alteration of these transcripts involved in endocrine signaling (*esr1, esr2b, vtg2*), and the ABC-transporter *abcc2* alteration, as well as *arnt2* alteration seem to be of minor importance.

3. Discussion

This study demonstrates the interference of CP1020 with many biological and physiological processes in zebrafish at the molecular level. Global transcriptome analysis revealed hitherto unknown mode of actions of this cyanopeptolin. Among many others, the most affected pathways were DNA damage recognition and repair, circadian rhythm, response to light and heme metabolism. Results achieved in this study give first insights into the possible modes of action of CP1020. Forthcoming investigations are needed to further analyse effects on the physiological level based on theses transcriptomics data. Exposure to two different CP1020 concentrations resulted in transcriptional alteration of many genes. 68 transcripts, were altered in both concentrations and except one, they were all regulated in the same direction when compared between the two concentrations, meaning each gene was either up-regulated in both concentrations, or down-regulated in both concentrations. However, a higher number of genes was altered only in one of the two concentrations. The two top-scored altered pathways, by analyzing transcripts that are altered in both dose groups, were DNA damage pathways involving the transcription factors and tumor suppressor genes *Brca1* and *Brca2*, which, however, are lacking in fish. Metacore analysis is based on the human genome, and therefore data from zebrafish are translated to human homologs or orthologs by this software. Pathway analysis is therefore related to humans, which indicates some restrictions to data interpretation for zebrafish (Fent and Sumpter, 2011).

Nevertheless, transcripts of important genes involved in DNA damage recognition and repair were differentially expressed at both CP1020 concentrations. The *Xeroderma pigmentosum*

297 complementation group C gene (*xpc*), and the damage-specific DNA binding protein 2 gene (*DDB2*)
298 were down-regulated (Table S2). Both genes are responsible for initiation of nucleotide excision repair
299 (NER), a system that eliminates a wide variety of helix-distorting DNA lesions (Matsuda *et al.*, 2005,
300 Araki *et al.*, 2001). XPC is a key factor in NER and together with *DDB-2* recruiting machinery to
301 eliminate DNA damage (Ray *et al.*, 2013). Defective XPC function results in a cancer prone
302 phenotype. A crucial role in preventing cancer is known for p53 and its activity seems to be affected
303 by CP1020 treatment. P300, activating p53 by covalent modification, was up-regulated at 1000 µg/L
304 (Table S4), while dual specificity phosphatase MKP1 (*dusp-1*), which plays a role in activation of p53
305 by inhibition of a p53 activating factor**,** that was significantly down-regulated at 1000 µg/L CP1020
306 (Table S4). The strong down regulation of *cry5*, the 6-4 photolyase in zebrafish at both CP1020
307 concentrations is further evidence for affected DNA damage repair by CP1020 (Figure 3). Photolyases
308 repair DNA adducts induced by UV-light (Sancar, 2003).

309 The circadian rhythm is regulated by a complex interaction of transcription-translation and
310 posttranslational feedback loops. They consist of core feedback loop genes (Clock and Bmal
311 heterodimers that regulate transcription of Period *per*), cryptochrome (*cry*) genes, and a stabilizing
312 loop (Rev-ERBalpha (*nr1d1*) and Rora regulate expression of the Clock and Bmal genes) (Vatine *et*
313 *al.*, 2011). In CP1020 exposed zebrafish eleuthero-embryos *nr1d1* was strongly down-regulated at
314 both concentrations (Figure 3), while *bmal1b* was slightly up-regulated (FC (log2) 0.8) at 1000 µg/L
315 (data not shown). Furthermore, the transcriptional repressor genes *per1a /per1b, nr1d2a* and *nr1d2b*
316 were significantly down-regulated at the high dose (Table S4). Transcripts of *per2a*, also acting as
317 transcriptional repressor, were slightly down-regulated (0.8-fold (log2)) in both dose groups. In
318 contrast to these down-regulated transcripts involved in the circadian rhythm, most of the transcripts
319 of opsin genes, *rho*, *opn1mw2*, *opn1sw2*, *opnsw1*, *tmtospa* were significantly or slightly up-regulated
320 at 100 µg/L (Tables S2, S3). Their expression is also regulated by the circadian rhythm (Li *et al.*,
321 2005) and esome genes involved in the regulation of the circadian rhythm are inducible by light, like
322 the D box-binding factor *TEF*, which directs light-induced clock gene expression (Gavriouchkina *et*
323 *al.*, 2010). Expression of *tef* was strongly down-regulated at 1000 µg/L (Table S4), and slightly (FC

0.6 (log2)) at 100 µg/L, suggesting that deregulation of circadian rhythm could be influenced by response to light.

Interestingly, in addition to *tef*, other light -inducible genes (cry5, cry-*DASH*, *per2*, si:ch211-195b13.1 , zgc:56136, zgc:153154, serum/glucocorticoid regulated kinase 1-like, *xpc*, *ptgds* among others) were also significantly down-regulated by CP1020 (Tables S2, S3, S4). The comparison of our data with the light responsive transcriptome of zebrafish (Weger *et al.*, 2011) suggests that CP1020 negatively influences the reaction onto light stimulus. Furthermore, the top-scored GO process by analysis of transcripts that are only altered in the low dose group was the neurophysiological process "visual perception" (data not shown). Transcriptional processes within the retina involved in visual perceptions were shown to be regulated by *nr1d1* (Mollema *et al.*, 2011) and *nr1d1* was strongly down-regulated in our study. However, in zebrafish the central photoperceptive organ is the pineal gland. The circadian rhythm controls a variety of cellular and physiological processes. Therefore, it seems likely that deregulation of the internal clock has further influence on additional pathways and processes, including hormonal pathways. Prostaglandin D2 is known to mediate sleep, body temperature and hormone release (Mong *et al.*, 2011) and prostaglandin D2 syntethase D2 (*ptgds*) is down-regulated, as shown by microarrays and validated by RT-qPCR. Furthermore, the top scored pathway at 1000 µg/L CP1020 was the Gonadotropin releasing hormone (GnRH) signaling and GnRH is a key hormone related to reproduction in vertebrates. Moreover, significant up-regulation of the estrogen receptor *esr1* at 100 µg/L, down-regulation of *esr2b* and vitellogenin (*vtg2*) up-regulation at 1000 µg/L were shown by microarray data. However this could only partly verified by RT-qPCR (for *esr1*). .

The ABC transporter *abcg2a* was altered in both doses (Figure 3). *Abcg2a* is important in heme transport (Desuzinges-Mandon *et al.*, 2011), and influence on heme metabolism was also shown by Metacore pathway analysis. Alteration of another ABC transporter, *abcc2,* also showed by microarray data, could not be confirmed by RT-qPCR, and thus, seeming to be of minor importance.

Exposure of zebrafish to CP1020 affected transcriptional expression of genes belonging to many different pathways. However, it should be noted that only a few transcripts of the affected pathways were altered, and only a relatively small number of transcripts (68 out of 484) showed similar

352 alterations in the low and high dose group. Pathway analyses showed distinct differences in the low
353 and high dose groups.
354 The global transcription profile clearly differed from that of MC-LR (Rogers *et al.*, 2011) and only a
355 few transcripts altered in common with *Microcystis* treatment (Rogers *et al.*, 2011). Of the nuclear
356 receptor gene family *nr1d2b,* was significantly down-regulated at 1000 µg/L CP1020 (Table S4), as
357 well as by *Microcystis* extracts (Rogers *et al.*, 2011), whereas *nr1d1* was strongly down-regulated at
358 both CP1020 concentrations (Figure 3), but not by *Microcystis* treatment. Transcripts altered by both
359 *Microcystis* and CP1020 treatment are transcripts of opsin genes, of krueppel like factor (however,
360 another isoform, and only in the high CP1020 dose group) and the thyrotroph embryonic factor *tef*
361 (both function in cell signaling and development) and vitellogenin *vtg* . However, CP1020 induced a
362 different *vtg* isoform, the *vtg II* transcript at 1000 µg/L CP1020 (Table S4), and induction was only six
363 times which is rather low compared to an induction of more than 100-times found by *Microcystis*
364 treatment (Rogers *et al.*, 2011). Furthermore induction by CP1020 could not be confirmed by RT-
365 qPCR. Taken together, the transcriptional profiles of MC-LR and CP1020 are distinct, and only a few
366 of the transcriptional alterations induced by *Microcystis* extracts (Rogers *et al.*, 2011) are also altered
367 by CP1020. The effects of CP1020 found in our study occur at two concentrations, which are assumed
368 to be rather high, but actual concentrations of CP1020 in surface or drinking water are currently
369 unknown. Field sampling would give some indication for the effective environmental concentration.
370 However, cyanopeptolins are widely distributed, not only produced by *Microcystis* species, and there
371 is a high structural variability among them.

372 In conclusion, we demonstrate that the novel cyanobacterial toxin CP1020 has important
373 transcriptional effects in zebrafish eleuthero-embryos altering a large number of transcripts. Global
374 transcriptome analysis revealed molecular effects and potential modes of action of this toxin, which
375 are distinct from those of MC-LR. However, the transcriptional response is complex and involves
376 many different albeit key biological and physiological processes. The most prominently affected
377 pathways were DNA damage recognition and repair, circadian rhythm, response to light, and to some
378 extent metabolic activities. All of them imply important ecological consequences including
379 neurotoxicity to fish feeding on cyanopeptolin-producing cyanobacteria and human health

consequences when drinking contaminated water. Further investigations should focus in detail on the human health and ecological implications of cyanopeptolins. In particular, further investigations should demonstrate as to what extent the hypothetical modes of action on the transcription levels translates to physiological effects in fish feeding on cyanopeptolin-containing cyanobacteria.

Supplementary Material

Table S1. Primers used for qPCR. Table S2. Significantly altered transcripts in at both concentrations (100 µg/L and 1000 µg/L CP1020) in common in zebrafish eleuthero-embryos (GeneSpring GX 11 adjusted $p<0.05$ and adjusted Fold Change absolute ≥2). Table S3. Significantly altered transcripts at 100 µg/L CP1020 in zebrafish eleuthero-embryos (GeneSpring GX 11 adjusted $p<0.05$ and adjusted Fold Change absolute ≥2). Table S4. Significantly altered transcripts at 1000 µg/L CP1020 in zebrafish eleuthero-embryos (GeneSpring GX 11 adjusted $p<0.05$ and adjusted Fold Change absolute ≥2). Table S5. Effects of CP1020 compared to effects of Cyclosporin A (CsA). Figure S1. Chromatogram of absorption (upper panel) and masses (lower panel) of CP1020 detected by LC-ESI-MS (liquid chromatography coupled to electrospray ionization mass spectrometry). Figure S2. Relative gene expression of *esr1* (A), *esr2b* (B), *abcc2* (C) and *arnt2* (D) in zebrafish eleuthero-embryos exposed to 100 µg/L and 1000 µg/L of CP1020, compared to embryos exposed to the solvent control (0.01%DMSO).

Acknowledgements

We thank the Functional Genomics Centre, ETH and University of Zürich, for microarray processing. This study was funded by the Swiss National Science Foundation (grant No. PDFMP3_132466 to K.F).

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Tables

Table 1. Nominal and mean values of determined CP1020 concentrations in fish media at the beginning (0 h) and end (96 h) of the experiment.

Nominal [µg/L]	**Measured [µg/L]**	
	0 h	96 h
DMSO control	0	0
100	128.1 ± 7.8	90.1 ± 33.0
1000	1185 ± 13.8	856 ± 57.3

532 **Table 2.** 10 top scored pathways (a), and 10 top GO processes (b) differentially expressed in common

533 at both CP1020 concentrations as represented in MetaCore (FDR< 0.05).

534 a.

Top Pathways	***p*Value**
DNA damage_Brca1 as a transcription regulator	1.055e-02
DNA damage_Role of Brca1 and Brca2 in DNA repair	1.055e-02
DNA damage_Nucleotide excision repair	1.266e-02
Transcription_P53 signaling pathway	1.371e-02
Neurophysiological process_Circadian rhythm	1.650e-02
Heme metabolism	3.489e-02
Putative pathways for stimulation of fat cell differentiation by Bisphenol A	1.000e+00
Development_EGFR signaling pathway	1.000e+00
Chemotaxis_CCL2-induced chemotaxis	1.000e+00
Immune response_Regulation of T cell function by CTLA-4	1.000e+00

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537 b.

Top GO processes	***p*Value**
Nucleotide-excision repair, DNA damage recognition	9.613e-04
Proline catabolic process to glutamate	1.442e-03
Proline catabolic process	1.922e-03
Negative regulation of toll-like receptor 4 signaling pathway	2.402e-03
Glutamate biosynthetic process	2.881e-03
Porphyrin-containing compound catabolic process	3.361e-03
Tetrapyrrole catabolic process	3.361e-03
Heme catabolic process	3.361e-03
Oositive regulation of cholesterol homeostasis	3.361e-03
Pigment catabolic process	3.361e-03

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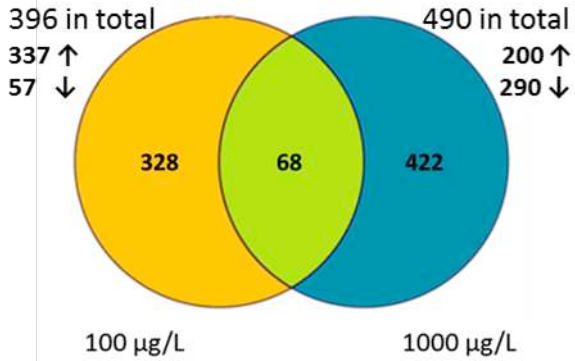
Figure Legends

Figure 1. Venn diagram showing the number of genes that are differentially expressed (fold change≥ 2, $p \leq 0.05$) in the respective treatment group relative to DMSO (0.01%) control. Shown are also the number of up-regulated and down-regulated transcripts. The overlapping region represents the number of genes (68) that are altered in common at both CP1020 concentrations.

Figure 2. Cluster analysis of all present genes in each replicate (n=4) from the different exposure groups.(Control: solvent control (0.01% DMSO); CP1020 low dose: 100 µg/L CP1020; CP1020 high dose: 1000 µg/L CP1020).

Figure 3. Relative transcriptional expression (shown as fold change log2) analyzed by microarray and RT-qPCR of *nr1d1* (A), *per1a* (B), *cry5* (C) ,*ptgds* (D), *abcg2a* (E) *abcc2* (F), of esr2b (G), esr1 (H) and *arnt2* (I) in zebrafish eleuthero-embryos exposed to 100 µg/L and 1000 µg/L of CP1020, compared to embryos exposed to the solvent control (0.01%DMSO). Results are given as mean ± standard deviation (n=4 replicates per treatment). Significant alterations compared to solvent control are indicated by asterisks. (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$).

564 **Figure 1**



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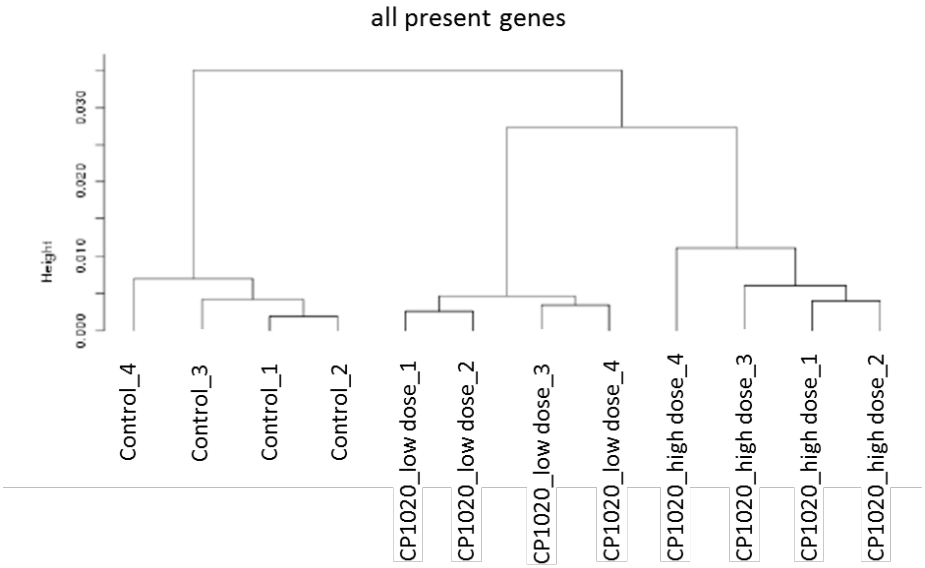
569 **Figure 2.**



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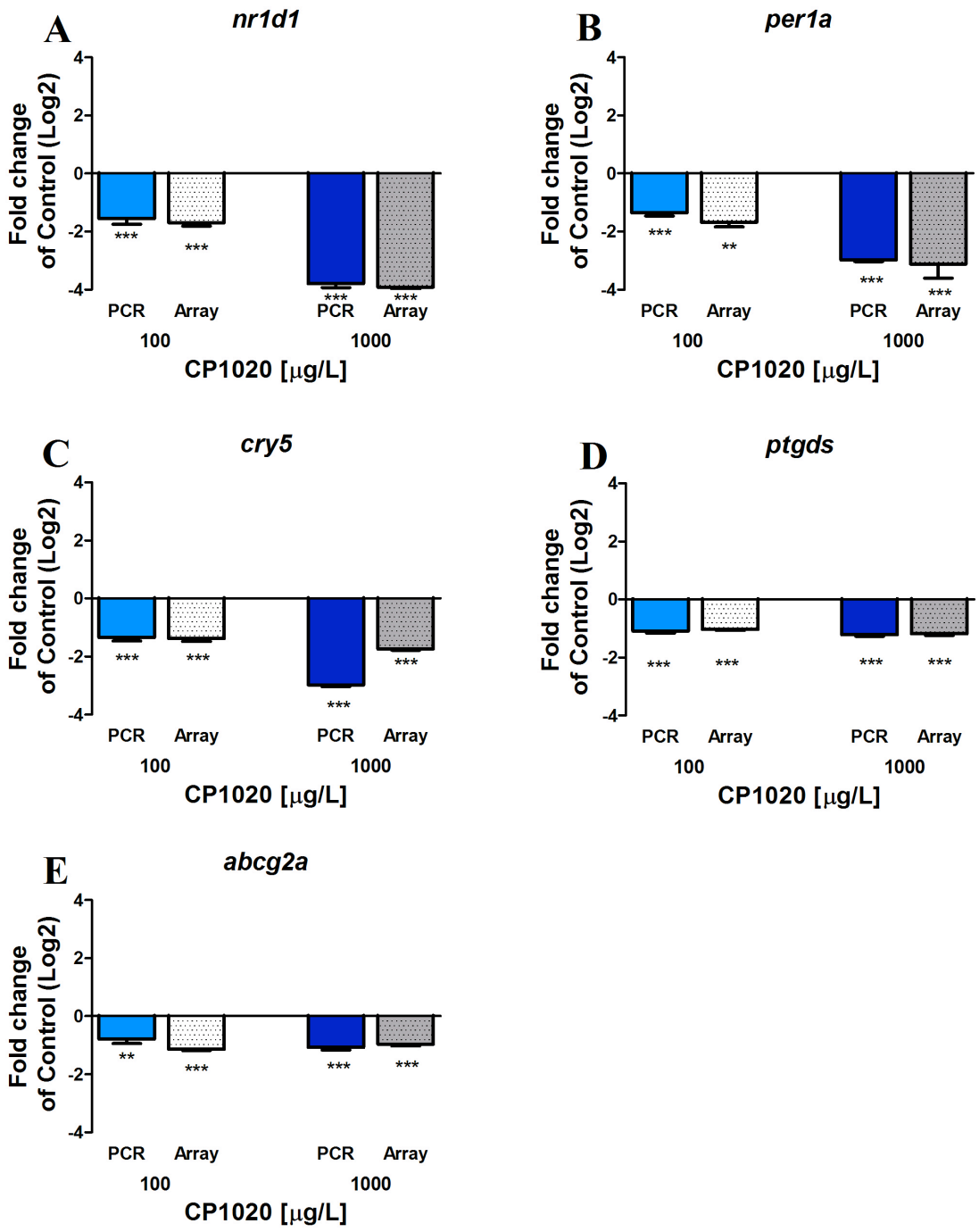
578 **Figure 3**



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Supplementary Information

Molecular effects of the cyanobacterial toxin cyanopeptolin (1020) occurring in algal blooms: global transcriptome analysis in zebrafish embryos

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Number of pages: 33

Number of tables: 4

Number of figures: 1

Supplementary information

LC-MS data of the purified CP1020 (Figure S1)

Figure S1: Chromatogram of absorption (upper panel) and masses (lower panel) of CP1020 detected by LC-ESI-MS (liquid chromatography coupled to electrospray ionization mass spectrometry). Purity was found >98% by absorption.

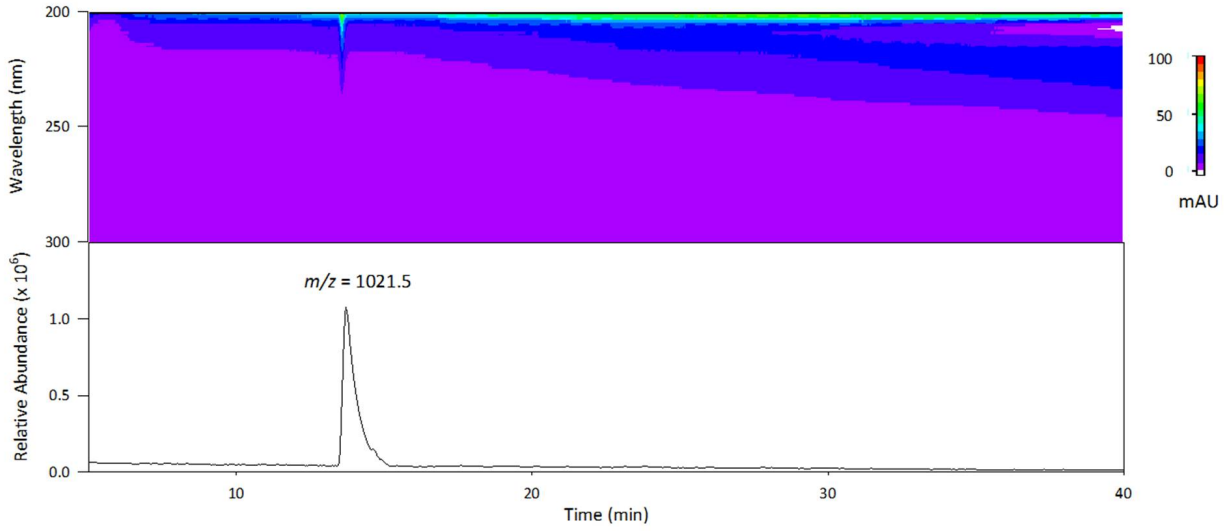
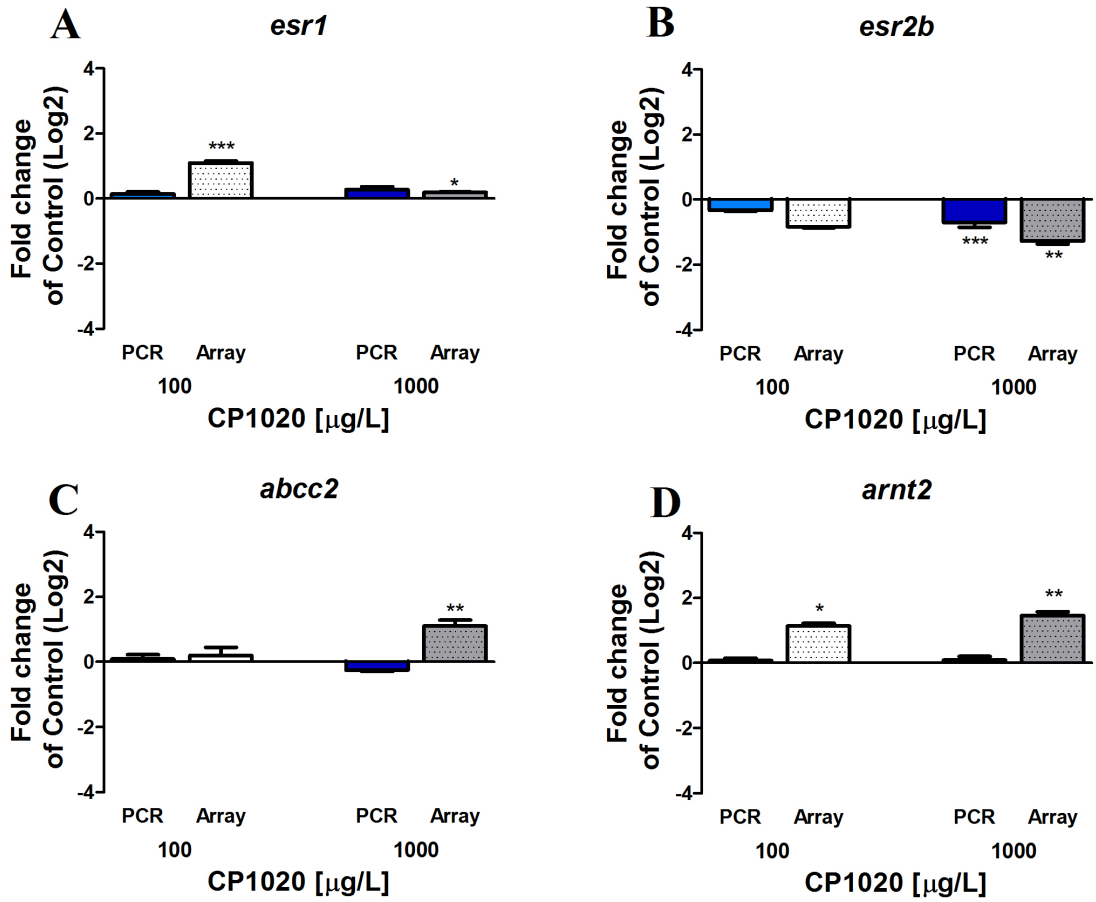


Figure S2

Relative gene expression of *esr1* (A), *esr2b* (B), *abcc2* (C) and *arnt2* (D) in zebrafish eleuthero-embryos exposed to 100 µg/L and 1000 µg/L of CP1020, compared to embryos exposed to the solvent control (0.01%DMSO). Results are given as mean ± standard deviation (n=4 replicates per treatment). Significant alterations compared to solvent control are indicated by asterisks. (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$).



List of all primers used for validation (Table S1)

Table S1: Primers used for qPCR.

Target gene	Primer	Sequence (5' - 3')
cry 5 [a]	Forward	CAT GGA GAG AAC GAA CTG GG
	Reverse	GTG CAG ACA AGC AGC CGA AC
nr1d1 [b]	Forward	GTG AAC AAC CAG CTG CAG AA
	Reverse	ACT GTA AGG CCT GGA CAT GG
per1 [c]	Forward	ATG CGT GCA AGA AGT GGT G
	Reverse	ACG TCC TCA TTT AGC GGA CTC
Ptgds [d]	Forward	CCA TCA AGA CCA AAG GAG GA
	Reverse	TCC ATT TTG TGG AAG CAT GA
esr1 [e]	Forward	TGA GCA ACA AAG GAA TGG AG
	Reverse	GTG GGT GTA GAT GGA GGG TTT
vtg II [f]	Forward	GGT GAC TGG AAG ATC CAA G
	Reverse	TCA TGC GGC ATT GGC TGG
esr2b [g]	Forward	TGG TCA TGT GAA GGG TGC AA
	Reverse	GCC TGA CAG CTC TTG CGT CT
abcc2 [h]	Forward	CCT CAT CCA CTG AAG AAC CGA
	Reverse	GCA CAG CAT CAA GGG AAA CA
arnt2 [i]	Forward	CAC CTT TGG ATC ACA TCT CAT TG
	Reverse	TCA CCC TCC TTA GAC GGA CC
abcg2a [j]	Forward	TCA TGA AGC CGG GAC TGA AC
	Reverse	GCT CCG TCT ATC AGC ACC TC

[a, b, c] Oggier et al., 2012. RN 107

[d] Zucchi et al., 2011. record number 102

[f] Meng et al., 2010 endnote record number 101

[g] Levi et al., 2011. RN:104

[h] Long et al., 20011. RN106

[i] designed using ncbi primertool

[k] Beherendt et al., 2010. RN: 103

Differential gene expression in CP1020 expsosed zebrafish embryos compared to control. Alterations unique to the different dose groups (Table S3, S4) and transcripts differentially expressed in common in both dose groups (Table S2).

Table S2: Significantly altered transcripts at both concentrations (100 µg/L and 1000 µg/L CP1020) in common in zebrafish eleuthero-embryos (GeneSpring GX 11 adjusted $p<0.05$ and adjusted Fold Change absolute ≥ 2).

Agilent ID	Gene Symbol	GenBank Accession	Regulation	Fold change log2 transformed	
				100 µg/L	1000 µg/L
A_15_P117046	opn1mw1	[NM_131253]	up	1.24	1.35
A_15_P100330	zgc:113054	[NM_001013450]	down	1.12	1.16
A_15_P406095		[TC396958]	down	1.82	2.55
A_15_P202856		[BC085364]	up	2.03	1.27
A_15_P207166	tmx3	[NM_001020557]	up	1.10	1.06
A_15_P621431	zgc:101803	[NM_001006051]	down	1.16	1.28
A_15_P197726	plek2	[NM_001128739]	down	1.16	1.48
A_15_P365290	fkbp5	[NM_213149]	down	1.39	3.56
A_15_P573722	glrx5	[NM_213021]	down/up	2.47	1.24
A_15_P228131	zgc:113054	[NM_001013450]	down	1.13	1.07
A_15_P139731	zgc:162180	[NM_001089446]	down	1.33	1.73
A_15_P104868		[TC455374]	down	2.12	3.00
A_15_P753176		[TC396958]	down	1.66	2.40
A_15_P598037	wu:fb14d10	[AI385043]	down	1.09	1.24
A_15_P119413	opn1sw2	[NM_131192]	up	1.08	1.10
A_15_P443450		[XM_688722]	down	1.36	1.27
A_15_P144496	wdr76	[NM_001198567]	down	1.64	1.82
A_15_P765751	odc1	[NM_131801]	down	1.17	1.70
A_15_P206101	si:ch211-132b12.7	[NM_001045055]	down	1.07	1.54
A_15_P376790	si:ch211-225b11.1	[NM_001110461]	down	1.04	1.11
A_15_P187316	nr1d1	[NM_205729]	down	1.96	4.03
A_15_P599667	wu:fb99e06	[NM_001202415]	up	2.18	2.35
A_15_P103946	cry5	[NM_131788]	down	1.37	1.74
A_15_P724691	zgc:56136	[NM_200198]	down	1.44	1.77
A_15_P165016	or128-3	[NM_001128416]	up	1.01	1.03
A_15_P104880	kera	[NM_001025548]	up	1.04	1.04
A_15_P656931	zgc:77060	[NM_001002218]	down	1.01	1.87
A_15_P118515	ptgds	[NM_213634]	down	1.03	1.19

A_15_P100682	zgc:92040	[NM_001002391]	up	1.58	2.37
A_15_P119682	zgc:153046	[NM_001076601]	down	1.21	1.57
A_15_P664016	arnt2	[AF219987]	up	1.14	1.46
A_15_P208526	zgc:153679	[NM_001076557]	down	1.26	1.62
A_15_P296716	tspo	[NM_001006032]	down	1.03	1.10
A_15_P466175	si:ch211-132b12.7	[NM_001045055]	down	1.28	1.23
A_15_P725141	zgc:158404	[NM_001080565]	up	1.23	1.30
A_15_P262636	wu:fc76c11	[AL722213]	up	1.29	1.28
A_15_P203161		[EH438032]	up	1.77	1.84
A_15_P177056		[BC107500]	down	1.09	1.80
A_15_P631266	nostrin	[NM_001113604]	up	1.55	1.26
A_15_P115391	crtac1a	[NM_001080178]	down	1.38	1.36
A_15_P374365	wu:fc17c12	[AW171599]	up	1.54	1.48
A_15_P110290	nr1d1	[NM_205729]	down	1.44	3.80
A_15_P673246			up	1.37	2.57
A_15_P278721	bcl2l13	[NM_001044891]	down	1.12	1.37
A_15_P153321	opn1mw1	[NM_131253]	up	1.36	1.38
A_15_P119024	crygm3	[NM_001007786]	up	1.53	1.14
A_15_P491692		[TC424418]	down	1.68	2.30
A_15_P117906	zgc:56136	[NM_200198]	down	1.28	1.53
A_15_P518177	si:rp71-1g18.5	[AI793430]	up	1.94	1.06
A_15_P134231	xpc	[NM_001045210]	down	1.01	1.28
A_15_P268376			up	1.16	1.40
A_15_P544372	cry5	[NM_131788]	down	1.40	1.66
A_15_P121196		[TC434049]	down	1.01	1.62
A_15_P744906	per1a	[NM_001030183]	down	1.68	3.12
A_15_P394265		[TC377322]	up	1.30	1.58
A_15_P573082	zgc:101803	[NM_001006051]	down	1.04	1.14
A_15_P187771		[XM_685174]	down	1.25	1.42
A_15_P259156		[GU012647]	down	1.18	1.58
A_15_P363565		[TC367727]	down	1.12	1.80

A_15_P171336	xpc	[NM_001045210]	down	1.32	1.41
A_15_P673721			down	1.12	1.49
A_15_P348955	ddb2	[NM_001083061]	down	1.03	1.13
A_15_P299196		[BC154035]	down	1.32	1.58
A_15_P214381	zgc:153046	[NM_001076601]	down	1.12	1.43
A_15_P346285	zgc:77060	[NM_001002218]	down	1.06	1.50
A_15_P145966		[TC374112]	down	1.18	2.22
A_15_P685026		[XM_001332159]	down	1.37	2.75
A_15_P412285			down	1.42	1.69

Table S3: Significantly altered transcripts at 100 µg/L CP1020 in zebrafish eleuthero-embryos (GeneSpring GX 11 adjusted $p<0.05$ and adjusted Fold Change absolut ≥2). Fold changes in the table are log2 transformed.

Agilent ID	Gene Symbol	GenBank Accession	Regulation	Fold change log2 transformed
				100 µg/L
A_15_P107037	abcc2	[NM_200589]	up	1.10
A_15_P212811	abcg2a	[NM_001042775]	down	1.14
A_15_P139691	acer1	[NM_001017603]	up	1.04
A_15_P754826	acta1b	[NM_214784]	up	1.28
A_15_P109735	aldh2b	[NM_213301]	up	1.40
A_15_P743276	arhgap12a	[NM_001126407]	up	2.24
A_15_P176841	arhgef7a	[BC095868]	up	1.07
A_15_P429265	arpc5a	[NM_194378]	up	1.06
A_15_P195536	asb11	[NM_214792]	up	1.01
A_15_P620201	asf1ba	[NM_207063]	up	1.28
A_15_P598312	atcaya	[AL723106]	up	1.30
A_15_P629036	atxn7l3	[NM_001040341]	up	1.26
A_15_P153341	bach1	[NM_001020663]	up	1.18
A_15_P391713	bco2b	[AW280014]	up	2.33
A_15_P118204	bdnf	[NM_131595]	up	1.02
A_15_P121044	c1d	[NM_001007059]	up	1.15
A_15_P593872	c6ast1	[NM_001043319]	up	1.05
A_15_P632226	ccbe1	[NM_001163923]	up	1.80
A_15_P710766	ch1073-291c23.1	[EH612477]	up	1.18
A_15_P148131	cnga5	[NM_001044746]	up	1.14
A_15_P103195	cnpy4	[NM_001039513]	up	1.19
A_15_P193571	crb3a	[NM_001045322]	up	1.10
A_15_P119272	crygm2b	[NM_001020783]	up	2.15
A_15_P109667	crygm2c	[NM_001007783]	up	2.18
A_15_P677171	crygm2d16	[CK353409]	up	1.92
A_15_P663971	cth1	[NM_130939]	up	2.15
A_15_P172931	cx41.8	[NM_001034988]	up	1.06
A_15_P625966	cyt1l	[NM_001082882]	up	1.38
A_15_P720286	dclre1c	[NM_001045101]	up	1.44

A_15_P731896	ddc	[NM_213342]	up	1.61
A_15_P427760	dgcr8	[NM_001122749]	up	1.02
A_15_P109699	dlgap5	[NM_001004592]	up	1.52
A_15_P193351	dnm2l	[NM_213242]	up	1.46
A_15_P751421	eif4a1b	[BC153507]	up	1.55
A_15_P726211	eomesb	[NM_001083575]	up	1.06
A_15_P211001	ercc3	[NM_201582]	up	1.40
A_15_P149356	esr1	[NM_152959]	up	1.10
A_15_P665661	fam203a	[BC154198]	up	1.12
A_15_P152846	fech	[NM_131631]	down	1.01
A_15_P113403	fgfr1a	[NM_152962]	up	1.06
A_15_P664161	fshr	[AY278107]	up	1.36
A_15_P664086	ftr23	[AM941338]	up	1.10
A_15_P699426	gad2	[NM_001017708]	up	1.09
A_15_P751896	gad2	[NM_001017708]	up	1.10
A_15_P657016	gatsl3	[NM_001020815]	up	1.36
A_15_P473105	ghdcl	[NM_001089358]	down	1.01
A_15_P173846	gk5	[NM_001077803]	up	1.10
A_15_P111532	gnb3b	[NM_213202]	up	1.01
A_15_P101751	gnmt	[NM_212816]	up	1.54
A_15_P176561	gpd1a	[NM_001017709]	up	1.02
A_15_P163556	gripap1	[BC129452]	up	1.28
A_15_P154256	grnas	[NR_003147]	up	1.35
A_15_P170106	guca1b	[NM_131871]	up	1.16
A_15_P105950	guca1e	[NM_200656]	up	1.07
A_15_P112225	hapln3	[NM_213101]	up	1.06
A_15_P134791	hey1	[NM_212561]	up	1.01
A_15_P112260	hnf4b	[NM_205546]	up	1.44
A_15_P263076	igiv1s5	[BC116528]	up	1.06
A_15_P556147	im:7148382	[BC091989]	up	1.04
A_15_P659711	irf11	[NM_205747]	up	1.04
A_15_P460395	itga11a	[NM_001172627]	up	1.28

A_15_P176926	itih3	[BC097018]	up	1.10
A_15_P661016	kctd9	[NM_001002738]	up	1.08
A_15_P614894	kras	[NM_001003744]	up	1.66
A_15_P620681	lgals1l1	[BC164225]	up	1.12
A_15_P568622	LOC100003140	[BC092129]	up	1.10
A_15_P682751	LOC100003937	[XM_001343335]	up	1.08
A_15_P492397	LOC100004186	[XM_001343513]	up	1.18
A_15_P232691	LOC100150968	[XM_001922707]	up	1.08
A_15_P767926	LOC100330526	[BC167473]	up	1.19
A_15_P685391	LOC100330610	[XR_084344]	up	1.46
A_15_P133496	LOC402804	[BC045899]	up	1.40
A_15_P654836	LOC560609	[NM_001145763]	up	1.24
A_15_P751561	LOC561143	[NM_001044954]	up	1.06
A_15_P145841	LOC566487	[NM_001127514]	up	1.22
A_15_P240501	LOC566570	[CK027807]	down	1.19
A_15_P121419	LOC571647	[NM_001109718]	up	1.20
A_15_P651747	LOC794656	[NM_001128355]	up	1.70
A_15_P624851	LOC796180	[NM_001128270]	up	1.03
A_15_P378310	LOC796981	[NM_001135982]	up	1.12
A_15_P107810	lyn	[NM_001004543]	up	1.25
A_15_P163871	mettl21d	[NM_001045345]	up	1.09
A_15_P140136	myl2	[NM_001040045]	up	1.04
A_15_P516772	ndufb6	[CK029482]	up	1.44
A_15_P133209	nop10	[AY648784]	up	2.04
A_15_P107850	nt5e	[NM_200932]	up	1.04
A_15_P130421	ogfod1	[NM_199689]	up	1.20
A_15_P208001	olfcw1	[NM_001018147]	up	1.29
A_15_P544897	opn1mw2	[NM_182891]	up	1.28
A_15_P623051	pi4kab	[NM_001113342]	up	1.58
A_15_P104724	pkd2	[NM_001002310]	up	1.65
A_15_P110904	pla2g6	[NM_213097]	up	1.11
A_15_P664571	plrg1	[NM_213440]	up	1.23

A_15_P673791	psmb4	[EV756382]	down	1.64
A_15_P113039	psmb8	[NM_131392]	up	1.35
A_15_P120823	psmg1	[NM_001002714]	up	1.64
A_15_P181876	pygo2	[NM_001033111]	up	1.57
A_15_P105967	rfc3	[NM_201457]	up	1.93
A_15_P140041	rhd	[NM_001024819]	up	1.10
A_15_P757506	rho	[NM_131084]	up	1.57
A_15_P103970	rilpl2	[BC081605]	up	1.23
A_15_P630501	rps28	[NM_213034]	up	1.52
A_15_P620181	rrs1	[NM_200062]	up	1.12
A_15_P172886	saga	[NM_200559]	up	1.42
A_15_P731381	sb:cb55	[CK708916]	up	1.41
A_15_P743678	shq1	[NM_001080600]	up	1.32
A_15_P688567	si:busm1-116l04.2	[BI880833]	up	1.18
A_15_P554612	si:busm1-180g5.6	[CO360697]	up	1.21
A_15_P465235	si:ch211-132a5.3	[AW154554]	up	1.00
A_15_P390110	si:ch211-232m7.1	[BC154428]	up	1.66
A_15_P206146	si:ch211-234p6.13	[NM_001025297]	up	1.03
A_15_P163771	si:ch211-262h13.5	[NM_001045212]	up	1.10
A_15_P254751	si:ch211-286e11.3	[DV600115]	up	1.38
A_15_P621066	si:ch211-5k11.6	[NM_001013461]	up	1.68
A_15_P264401	si:dkey-165a24.3	[CK024736]	up	1.17
A_15_P139991	si:dkey-266f7.4	[NM_001100018]	up	1.24
A_15_P722281	si:dkey-37o8.1	[NM_001163994]	up	1.73
A_15_P594197	si:dkey-42o15.2	[CK362617]	up	1.86
A_15_P206201	si:dkey-53p21.1	[NM_001017734]	up	1.24
A_15_P406115	si:dkeyp-13f6.4	[BC142817]	up	1.45
A_15_P107681	slc2a2	[NM_001042721]	up	1.46
A_15_P662621	slc35d1b	[NM_200475]	up	1.12

A_15_P119354	smarca4	[BC066372]	up	1.55
A_15_P626791	spon2a	[NM_131007]	up	1.14
A_15_P403795	sst1.1	[CT663858]	up	1.22
A_15_P466565	stam	[NM_200120]	up	1.12
A_15_P114034	supt3h	[NM_001002625]	up	3.06
A_15_P133441	sypl2a	[BC044419]	up	1.79
A_15_P638399	tk1	[NM_199832]	up	1.30
A_15_P396715	tlcd2	[NM_001110382]	down	1.04
A_15_P175326	tmem167a	[NM_001023581]	up	1.06
A_15_P458110	tmem176l.4	[NR_023331]	up	1.48
A_15_P183171	tnnc1a	[NM_181498]	up	1.16
A_15_P100535	traf4b	[NM_212817]	up	1.33
A_15_P707861	tuba4l	[NM_199795]	up	1.04
A_15_P625186	tuba7l	[NM_001002230]	up	1.30
A_15_P719736	ubtfl	[NM_201003]	up	1.10
A_15_P148711	ubxn10	[NM_001045446]	up	1.38
A_15_P620511	vti1a	[NM_001034980]	up	1.04
A_15_P596387	wu:fa01c11	[AA495404]	up	1.28
A_15_P772671	wu:fa04a09	[CK028742]	up	1.21
A_15_P673511	wu:fa04f03	[CN020186]	up	1.33
A_15_P402380	wu:fa04h02	[CN506356]	up	2.32
A_15_P402480	wu:fa05f02	[CK030220]	up	1.04
A_15_P673416	wu:fa12f04	[EE301576]	up	1.08
A_15_P597022	wu:fa14a01	[CT691680]	up	1.01
A_15_P673641	wu:fa14a04	[CT670778]	up	1.19
A_15_P462735	wu:fa98d09	[AI331404]	up	1.17
A_15_P261986	wu:fb08f10	[AI396676]	down	1.04
A_15_P462820	wu:fb12a02	[AI384722]	up	1.32
A_15_P328351	wu:fb39g11	[CT723881]	up	1.31
A_15_P673566	wu:fb49f03	[CK027957]	up	1.27
A_15_P596322	wu:fb51f10	[CN319073]	up	2.19
A_15_P706021	wu:fb58c03	[EE715876]	up	1.32

A_15_P402905	wu:fb74h05	[AI545174]	up	1.51
A_15_P429110	wu:fb77b09	[CT699080]	up	1.47
A_15_P696756	wu:fb79a03	[CT665841]	up	1.14
A_15_P596727	wu:fb97d04	[EL645854]	up	1.65
A_15_P516387	wu:fc01e05	[CT585687]	up	1.09
A_15_P517172	wu:fc06d12	[DN902971]	up	1.48
A_15_P516492	wu:fc09h01	[AI601332]	up	1.33
A_15_P449295	wu:fc11d12	[CT715359]	up	1.33
A_15_P407868	wu:fc22g10	[AI641705]	up	1.14
A_15_P687697	wu:fc26h06	[AL719858]	up	1.46
A_15_P452485	wu:fc31d10	[BM529384]	up	1.09
A_15_P517947	wu:fc32b03	[AI722292]	up	1.20
A_15_P518352	wu:fc55f12	[CT623879]	up	1.12
A_15_P516852	wu:fc72a10	[CT678105]	up	1.07
A_15_P535477	wu:fc88f10	[CT720201]	up	1.11
A_15_P262166	wu:fc96h04	[AI958591]	up	2.62
A_15_P682276	wu:fd11e12	[AW419602]	up	1.24
A_15_P612062	wu:fd11h09	[AI959616]	up	1.02
A_15_P518772	wu:fd21d09	[AI882800]	up	1.47
A_15_P696361	wu:fd60d11	[EH999512]	up	1.08
A_15_P773571	wu:fi33h02	[AW175245]	up	1.23
A_15_P392090	wu:fi74c02	[EH505571]	up	1.01
A_15_P592577	wu:fj02e11	[AW078029]	up	1.24
A_15_P263936	wu:fj54b05	[DV586416]	up	1.95
A_15_P756976	wu:fj78f12	[CF999436]	up	1.28
A_15_P612452	wu:fl03g02	[CT641231]	up	1.38
A_15_P101811	xrcc4	[NM_200786]	up	1.42
A_15_P671451	zdhhc6	[EG581243]	up	1.94
A_15_P629796	zgc:110304	[NM_001017593]	up	2.42
A_15_P663616	zgc:110425	[NM_001020746]	up	1.01
A_15_P162166	zgc:110697	[NM_001017864]	up	1.04
A_15_P163096	zgc:111893	[NM_001039986]	up	2.31

A_15_P205141	zgc:112266	[NM_001024437]	up	1.99
A_15_P626016	zgc:112368	[NM_001025474]	up	1.20
A_15_P176075	zgc:112374	[NM_001024417]	up	2.37
A_15_P171666	zgc:112484	[NM_001017668]	up	1.46
A_15_P195451	zgc:112982	[NM_001013349]	up	1.43
A_15_P635191	zgc:113984	[NM_001025176]	up	1.87
A_15_P153386	zgc:114123	[NM_001030232]	up	1.91
A_15_P160906	zgc:123274	[NM_001037378]	up	1.08
A_15_P150776	zgc:136683	[NM_001040311]	up	2.24
A_15_P760561	zgc:136683	[NM_001040311]	up	1.26
A_15_P208016	zgc:136758	[NM_001045300]	up	1.14
A_15_P620541	zgc:152936	[NM_001045417]	up	1.23
A_15_P170476	zgc:152945	[NM_001076754]	up	1.32
A_15_P744351	zgc:153084	[NM_001077578]	up	1.77
A_15_P661766	zgc:153092	[NM_001077335]	up	1.61
A_15_P757216	zgc:153431	[BC122290]	up	1.01
A_15_P100387	zgc:153846	[NM_001076649]	up	1.58
A_15_P283311	zgc:154110	[NM_001077543]	up	1.33
A_15_P472010	zgc:158225	[NM_001080580]	up	1.05
A_15_P620781	zgc:158657	[NM_001080645]	up	1.29
A_15_P163476	zgc:162213	[NM_001089474]	up	1.03
A_15_P120330	zgc:163091	[NM_001082833]	up	3.56
A_15_P195286	zgc:165507	[NM_001099419]	up	2.05
A_15_P151216	zgc:165526	[NM_001099258]	up	1.02
A_15_P162286	zgc:165543	[NM_001098765]	up	1.14
A_15_P321988	zgc:165582	[NM_001102636]	up	1.03
A_15_P628391	zgc:171482	[NM_001128272]	up	1.08
A_15_P733376	zgc:171667	[NM_001105702]	up	1.26
A_15_P271771	zgc:171837	[NM_001114573]	up	1.37
A_15_P254266	zgc:171957	[NM_001110169]	up	2.15
A_15_P621966	zgc:171965	[NM_001102624]	up	1.01
A_15_P627471	zgc:172075	[NM_001114408]	up	1.28

A_15_P701751	zgc:172106	[NM_001114563]	up	2.46
A_15_P627286	zgc:172215	[NM_001114567]	up	1.50
A_15_P728326	zgc:172225	[NM_001114901]	up	1.16
A_15_P616733	zgc:173575	[NM_001114921]	up	1.45
A_15_P775256	zgc:173617	[NM_001109839]	up	1.06
A_15_P774321	zgc:173619	[NM_001109740]	up	1.03
A_15_P665771	zgc:173726	[BC155251]	up	1.26
A_15_P110273	zgc:174160	[NM_001114421]	up	1.14
A_15_P755141	zgc:174164	[NM_001114439]	up	1.01
A_15_P452365	zgc:174263	[NM_001114314]	up	1.14
A_15_P623156	zgc:174653	[NM_001114894]	up	1.11
A_15_P666396	zgc:174689	[NM_001103131]	up	1.40
A_15_P113678	zgc:175088	[NM_001113798]	up	1.13
A_15_P183656	zgc:194189	[NM_001128810]	up	1.35
A_15_P691881	zgc:63882	[NM_200402]	up	1.15
A_15_P669901	zgc:73293	[CF549987]	up	1.47
A_15_P112827	zgc:92162	[NM_001002350]	up	1.15
A_15_P425695	zrsr2	[CT650759]	up	1.50
A_15_P417575	zwi	[NM_001159664]	up	1.35
A_15_P418985		[TC378307]	up	1.10
A_15_P290011			up	2.05
A_15_P547507			up	1.18
A_15_P118554		[TC386806]	up	1.56
A_15_P686381		[EE717155]	up	1.47
A_15_P499182		[XM_691353]	up	1.79
A_15_P257243			up	1.01
A_15_P575717		[BI897764]	up	1.41
A_15_P673351			up	1.05
A_15_P235101			down	1.13
A_15_P561117		[EH448530]	up	1.61
A_15_P681596		[TC399076]	up	1.04
A_15_P676196			up	1.68

A_15_P310441	[DV590206]	up	1.26
A_15_P686151		up	1.06
A_15_P674281	[TC427566]	up	1.72
A_15_P217721		up	1.11
A_15_P133936	[BC067712]	up	1.08
A_15_P224801	[CK015433]	up	1.04
A_15_P431355	[TC412525]	up	1.06
A_15_P577207	[CN019041]	up	2.04
A_15_P406085	[BQ259582]	up	1.08
A_15_P183636		up	1.14
A_15_P710011	[XM_001919397]	up	1.30
A_15_P297346	[TC387556]	up	1.13
A_15_P417785	[TC382901]	up	1.07
A_15_P610232	[BC096919]	up	1.11
A_15_P502757	[CT640649]	up	1.01
A_15_P764951		up	1.48
A_15_P759911		down	1.20
A_15_P611167	[BC150433]	up	1.29
A_15_P169926	[BC129502]	up	1.57
A_15_P385980	[CT627095]	up	1.25
A_15_P411618		up	1.73
A_15_P176821	[BC095808]	up	1.11
A_15_P268581		up	1.61
A_15_P259953	[XR_084489]	up	1.57
A_15_P400565		up	1.01
A_15_P486355	[TC390102]	up	1.24
A_15_P520762		up	1.23
A_15_P561152	[EH451159]	up	1.34
A_15_P109801	[BC125930]	up	1.20
A_15_P574140		up	2.50
A_15_P165373	[XM_001345533]	up	1.77
A_15_P178016	[BC129358]	up	1.10

A_15_P712206	[TC383262]	up	1.04
A_15_P458215		up	2.62
A_15_P113474	[BC142909]	up	1.03
A_15_P376595		up	1.34
A_15_P709716		up	1.29
A_15_P490517		up	1.03
A_15_P682801	[DN601273]	up	1.05
A_15_P710791	[EV757292]	up	1.94
A_15_P157951	[TC402912]	up	1.66
A_15_P684496	[TC421118]	up	1.88
A_15_P536027		up	1.14
A_15_P764036		up	1.67
A_15_P309131	[TC395274]	up	1.12
A_15_P279076	[XM_679191]	up	1.10
A_15_P483720	[TC400670]	up	1.29
A_15_P705201		up	1.18
A_15_P270601	[EH448452]	up	1.55
A_15_P138626	[XM_003201046]	up	1.85
A_15_P694736	[TC388621]	up	1.18
A_15_P490487	[CK030074]	up	2.00
A_15_P764326		up	1.62
A_15_P201421	[TC457003]	up	1.59
A_15_P179696	[BC152020]	up	1.64
A_15_P693526	[BC162656]	up	1.14
A_15_P186781		up	1.86
A_15_P758701	[EH538135]	up	1.91
A_15_P685476	[XM_691874]	up	2.17
A_15_P171121	[AB235997]	up	1.22
A_15_P762031		up	1.14
A_15_P398325		up	1.01
A_15_P138601		up	1.84
A_15_P601617	[CN500586]	up	1.16

A_15_P298486	[TC392290]	up	1.01
A_15_P168521	[TC440865]	up	1.21
A_15_P244746	[TC386891]	up	1.57
A_15_P761423		up	1.45
A_15_P397440		up	1.06
A_15_P404450	[BC150163]	up	2.16
A_15_P112552		up	1.30
A_15_P444770		up	1.84
A_15_P774997		up	1.12
A_15_P775096		up	1.21
A_15_P108727	[BC068409]	up	1.11
A_15_P762091		up	1.04
A_15_P116950	[BC076487]	up	1.05
A_15_P281941	[TC400642]	down	1.16

Table S4: Significantly altered transcripts at 1000 µg/L CP1020 in zebrafish eleuthero-embryos (GeneSpring GX 11 adjusted $p<0.05$ and adjusted Fold Change absolut ≥2). Fold changes in the table are log2 transformed.

Agilent ID	Gene Symbol	GenBank Accession	Regulation	Fold change log2 transformed
				1000 µg/L
A_15_P331136	abcf2	[NM_201315]	down	1.96
A_15_P153641	abhd2a	[NM_200914]	down	1.01
A_15_P141526	ankrd1b	[NM_001102388]	down	1.21
A_15_P721076	arl5c	[NM_200846]	down	1.16
A_15_P106879	arr3b	[NM_200792]	down	1.27
A_15_P668738	arr3b	[NM_200792]	down	1.20
A_15_P645026	arr3b	[NM_200792]	down	1.45
A_15_P141096	bhlhe41	[NM_001039107]	down	1.00
A_15_P103119	btg2	[NM_130922]	down	1.05
A_15_P535632	bzw1b	[NM_213092]	down	1.65
A_15_P658516	cbfb	[NM_199209]	up	1.23
A_15_P741541	ccna1	[NM_212818]	up	1.77
A_15_P113920	cebpb	[NM_131884]	down	1.07
A_15_P205136	cldnb	[NM_131763]	down	1.21
A_15_P504397	col11a1a	[NM_001083844]	up	1.36
A_15_P420630	coq10b	[NM_001017747]	down	1.05
A_15_P720981	cpa2	[NM_001003446]	down	1.26
A_15_P664221	cry5	[BC044204]	down	1.52
A_15_P120707	cry-dash	[NM_205686]	down	1.31
A_15_P105031	csrnp1b	[NM_199619]	down	1.26
A_15_P728106	csrnp1b	[NM_199619]	down	1.30
A_15_P186886	cyp2k18	[NM_200512]	up	1.59
A_15_P176516	dapp1	[NM_001017691]	down	1.31
A_15_P627076	dapp1	[NM_001017691]	down	1.29
A_15_P373660	dbp2	[NM_001197062]	down	1.27
A_15_P568047	dolk	[NM_001110484]	up	1.25
A_15_P116888	dusp1	[NM_213067]	down	1.21
A_15_P108530	dusp1	[NM_213067]	down	1.34
A_15_P658831	dusp2	[BC163999]	down	1.05
A_15_P723641	egr2a	[NM_183341]	down	2.39

A_15_P686291	egr2a	[NM_183341]	down	1.98
A_15_P119478	egr2a	[NM_183341]	down	2.17
A_15_P566792	eif2ak2	[NM_001114470]	up	1.34
A_15_P142261	elovl6l	[NM_201500]	down	1.21
A_15_P197591	elovl6l	[NM_201500]	down	1.22
A_15_P674886	elovl6l	[NM_201500]	down	1.13
A_15_P132441	esr2b	[NM_174862]	down	1.28
A_15_P193416	f2rl1.2	[NM_001098778]	down	1.57
A_15_P242841	f2rl1.2	[NM_001098778]	down	1.46
A_15_P596847	fabp2	[EH497825]	up	1.43
A_15_P103810	fam46bb	[NM_001045170]	down	1.18
A_15_P107943	fam46bb	[NM_001045170]	down	1.26
A_15_P114779	fgfrl1b	[NM_001012263]	up	1.14
A_15_P518977	filip1	[AI884079]	up	1.54
A_15_P695676	fkbp5	[NM_213149]	down	1.67
A_15_P104381	fos	[NM_205569]	down	1.33
A_15_P184851	fos	[NM_205569]	down	1.07
A_15_P729336	fos	[NM_205569]	down	1.23
A_15_P630281	fosb	[NM_001007312]	down	1.24
A_15_P767986	fosl2	[NM_001082998]	down	1.06
A_15_P740961	fosl2	[NM_001082998]	down	1.08
A_15_P397480	foxf1	[NM_001080186]	up	1.58
A_15_P767621	gch2	[NM_131667]	down	1.85
A_15_P738231	gch2	[NM_131667]	down	1.50
A_15_P419550	gch2	[NM_131667]	down	1.48
A_15_P172406	gch2	[NM_131667]	down	1.51
A_15_P178241	gngt2b	[NM_001204332]	up	1.10
A_15_P106583	hbbe3	[NM_001015058]	up	1.06
A_15_P110156	hells	[NM_001037101]	down	1.23
A_15_P160691	hells	[NM_001037101]	down	1.68
A_15_P198206	hig1	[NM_200100]	down	1.32
A_15_P443630	hspb6	[NM_001100958]	down	1.40
A_15_P371510	im:6912096	[EE689330]	up	2.83

A_15_P228786	im:7141374	[EH999121]	up	1.54
A_15_P103066	ing5a	[NM_198211]	down	1.19
A_15_P119303	irf11	[NM_205747]	up	1.16
A_15_P648216	itgb1bp3	[NM_001004618]	down	1.85
A_15_P114717	itgb1bp3	[NM_001004618]	down	1.76
A_15_P728736	itln3	[NM_001159584]	down	1.80
A_15_P199681	jund	[NM_001128342]	down	1.06
A_15_P737411	kcnk1	[NM_001098753]	down	1.00
A_15_P646491	kdm5bb	[NM_001002166]	up	1.54
A_15_P218661	klf2a	[NM_131856]	down	1.10
A_15_P108040	klf2a	[NM_131856]	down	1.01
A_15_P668111	klf4a	[NM_001113483]	down	1.57
A_15_P346495	klf4a	[NM_001113483]	down	1.58
A_15_P384005	klf4a	[NM_001113483]	down	1.34
A_15_P735206	klf9	[NM_001128729]	down	1.11
A_15_P146191	klf9	[NM_001128729]	down	1.16
A_15_P162489	lingo4a	[NM_001082978]	up	1.86
A_15_P110548	lipg	[NM_200128]	down	1.04
A_15_P200506	lmo7b	[NM_001045444]	up	1.34
A_15_P359121	LOC100003022	[XM_001342634]	down	1.82
A_15_P101230	LOC100005105	[BC139489]	up	1.06
A_15_P763171	LOC100534915	[XM_003200278]	up	1.50
A_15_P441850	LOC559147	[NM_001123245]	up	1.21
A_15_P590392	LOC567678	[EE713500]	up	1.18
A_15_P412195	LOC793143	[NM_001122706]	down	1.28
A_15_P577092	loxl2a	[NM_001099244]	down	1.18
A_15_P344837	lpin1	[NM_001044353]	down	1.26
A_15_P392640	lsm6	[EH511819]	up	1.06
A_15_P147321	mef2cb	[NM_001130962]	up	1.75
A_15_P117696	mfsd2ab	[NM_001003570]	down	1.29
A_15_P486995	mfsd2ab	[NM_001003570]	down	1.42
A_15_P265196	mg:ab01b07	[EH585820]	up	1.23
A_15_P705451	midn	[NM_207052]	down	1.04

A_15_P209266	mmp13a	[NM_201503]	down	1.21
A_15_P106403	msh6	[NM_182860]	up	1.12
A_15_P118680	mstna	[NM_001004122]	up	2.14
A_15_P115043	mt	[NM_131075]	up	1.16
A_15_P686380	mtr	[NM_198072]	down	1.11
A_15_P107751	mxa	[NM_182942]	up	1.21
A_15_P152006	mybpc1	[NM_001007322]	up	1.60
A_15_P369250	mych	[NM_001126109]	down	1.24
A_15_P263391	mylz3	[CF266090]	up	1.48
A_15_P720546	ncapd2	[NM_001162502]	up	1.02
A_15_P115542	neurod	[NM_130978]	up	1.11
A_15_P110814	nos2a	[NM_001104937]	up	1.78
A_15_P228446	nr1d2a	[NM_001130592]	down	1.97
A_15_P156006	nr1d2a	[NM_001130592]	down	1.57
A_15_P433255	nr1d2b	[NM_131065]	down	1.13
A_15_P659251	nr4a1	[NM_001002173]	down	1.83
A_15_P109764	nr4a1	[NM_001002173]	down	2.12
A_15_P174241	oat	[NM_207077]	down	1.11
A_15_P517612	odc1	[NM_131801]	down	1.35
A_15_P107148	opn1mw2	[NM_182891]	up	1.14
A_15_P158224	or109-6	[NM_001128409]	up	2.12
A_15_P721629	or136-1	[NM_001136247]	up	1.01
A_15_P135166	pdk2	[NM_200996]	down	1.22
A_15_P104502	per1b	[NM_212439]	down	1.29
A_15_P658736	per1b	[NM_212439]	down	1.59
A_15_P186271	pfkfb4l	[NM_198816]	down	1.50
A_15_P174861	pfkfb4l	[NM_198816]	down	1.24
A_15_P102138	pfkfb4l	[NM_198816]	down	1.24
A_15_P116114	pgp	[NM_212726]	down	1.51
A_15_P347630	pkp2	[NM_001113433]	up	1.29
A_15_P136766	pkp3	[NM_001045280]	down	1.28
A_15_P345880	pkp3	[NM_001045280]	down	1.03
A_15_P624466	pkz	[NM_001040376]	up	1.02

A_15_P740511	plod1a	[NM_001077742]	up	1.27
A_15_P627816	poc1a	[NM_213049]	up	1.06
A_15_P169931	pou4f3	[NM_131278]	down	1.14
A_15_P658686	prkri	[BC163418]	up	1.06
A_15_P510777	prrc1	[NM_001007060]	up	1.06
A_15_P681846	ptges3b	[DV591682]	up	2.92
A_15_P142661	rhcga	[NM_001089577]	down	1.08
A_15_P368110	ripply2	[CT663488]	up	1.26
A_15_P279906	rnasen	[NM_001110472]	up	1.07
A_15_P671221	rps7	[CF595273]	up	3.11
A_15_P620206	rtn4rl2a	[NM_203479]	down	1.10
A_15_P417175	sb:cb1050	[DV588535]	down	1.29
A_15_P729196	sb:cb1050	[DV588535]	down	1.24
A_15_P690211	sb:cb1050	[DV588535]	down	1.20
A_15_P670366	sb:cb118	[EH440970]	up	1.07
A_15_P674271	sb:cb930	[EH448781]	down	1.55
A_15_P106112	sec23b	[NM_199777]	down	1.01
A_15_P107078	serpine1	[NM_001114559]	down	1.25
A_15_P727836	sgk1	[NM_199212]	down	1.46
A_15_P131381	sgk1	[NM_199212]	down	1.28
A_15_P631896	sgk1	[NM_199212]	down	1.14
A_15_P601187	si:ch211-132b12.7	[NM_001045055]	down	1.63
A_15_P347300	si:ch211-225b11.1	[NM_001110461]	down	1.05
A_15_P625276	si:ch211-234p6.8	[NM_001128794]	up	1.96
A_15_P201276	si:ch211-235e18.3	[NM_001126383]	down	1.31
A_15_P229881	si:ch211-235e18.3	[NM_001126383]	down	1.07
A_15_P160641	si:ch211-264f5.2	[NM_001098253]	down	1.91
A_15_P182041	si:dkey-109n11.1	[NM_001197060]	down	1.32
A_15_P194771	si:dkey-11p23.7	[NM_001102395]	up	1.19

A_15_P683726	si:dkey-240e12.6	[EH519762]	up	1.89
A_15_P167546	si:dkey-30c15.2	[NM_001110381]	down	1.15
A_15_P133821	si:dkey-52h23.1	[NM_001197058]	up	1.21
A_15_P663181	si:dkey-91f15.1	[NM_001083108]	down	1.06
A_15_P175466	si:dkeyp-117h8.4	[NM_001082878]	up	1.58
A_15_P104774	si:dkeyp-59a8.1	[CN504135]	up	1.14
A_15_P199141	slc1a8b	[NM_001190816]	down	1.55
A_15_P749956	slc20a1b	[NM_212588]	down	1.06
A_15_P102197	slc20a1b	[NM_212588]	down	1.24
A_15_P236146	slc20a1b	[NM_212588]	down	1.18
A_15_P172956	slc22a7a	[NM_001083861]	down	1.12
A_15_P737561	slc25a14	[NM_200164]	down	1.38
A_15_P164941	slc25a15a	[NM_001080638]	down	1.02
A_15_P582067	slc25a21	[NM_001076632]	up	1.51
A_15_P101805	slc25a25a	[NM_213257]	down	1.64
A_15_P146426	slc25a25b	[NM_001160020]	down	1.23
A_15_P461950	slc25a33	[NM_213157]	down	1.60
A_15_P121224	slc25a33	[NM_213157]	down	1.20
A_15_P669271	slc25a38a	[NM_001076602]	down	1.01
A_15_P150506	slc2a11l	[NM_001080016]	down	1.48
A_15_P632826	slc2a5	[NR_023322]	up	1.06
A_15_P120123	slc34a2aas	[NR_002876]	up	1.16
A_15_P569107	slc35f3	[NM_001111205]	down	1.10
A_15_P196801	slc39a3	[NM_001080619]	up	1.21
A_15_P473660	slco2a1	[NM_001089582]	down	1.38
A_15_P174486	slmo2	[NM_199734]	down	1.59
A_15_P720506	slmo2	[NM_199734]	down	1.62
A_15_P275116	slmo2	[NM_199734]	down	1.81
A_15_P111231	snrk1	[NM_200833]	down	1.09
A_15_P394080	sst3	[CF550196]	up	1.18
A_15_P623556	stard10	[NM_200220]	down	1.02
A_15_P110631	sycp3l	[NM_001040350]	down	1.20

A_15_P629056	tef	[NM_131400]	down	1.02
A_15_P749236	tef	[NM_131400]	down	1.01
A_15_P166596	tef	[NM_131400]	down	1.04
A_15_P108054	tef	[NM_131400]	down	1.33
A_15_P108051	tlr1	[NM_001130593]	up	1.16
A_15_P432340	tmem144b	[NM_001005983]	up	1.62
A_15_P624091	traf4b	[NM_212817]	up	1.02
A_15_P729090	ugt5a2	[NM_001076723]	down	1.91
A_15_P205041	ugt5a2	[NM_001076723]	down	1.85
A_15_P111069	upp1	[NM_001013301]	down	1.20
A_15_P206701	ush1c	[NM_001039929]	down	2.03
A_15_P153366	vent	[NM_131700]	up	1.02
A_15_P205536	vgll4l	[NM_001079998]	up	1.02
A_15_P753881	vtg2	[NM_001110384]	up	2.76
A_15_P112367	wnt10b	[NM_178219]	up	1.08
A_15_P674261	wu:fb96d06	[CK706917]	up	1.74
A_15_P518022	wu:fc33a07	[AI878494]	up	1.13
A_15_P519572	wu:fd12h02	[AI959687]	down	2.16
A_15_P101169	wu:fe18c06	Danio rerio wu:fe18c06 (wu:fe18c06), mRNA [NM_001144810]	down	1.51
A_15_P591177	wu:fj66a01	[CO402997]	down	1.37
A_15_P672346	wu:fj67g09	[CD595504]	down	1.32
A_15_P262321	wu:fj68e06	[AW076550]	up	1.05
A_15_P264226	wu:fk20e09	[CT714447]	up	1.41
A_15_P596357	wu:fk45f12	[EG576070]	up	1.54
A_15_P640121	zgc:100911	[NM_001003641]	down	1.12
A_15_P624721	zgc:101565	[NM_001004641]	down	2.13
A_15_P103808	zgc:101565	[NM_001004641]	down	2.03
A_15_P728131	zgc:101724	[NM_001024098]	up	1.22
A_15_P455820	zgc:103654	[NM_001007363]	down	1.04
A_15_P143116	zgc:110200	[NM_001017825]	down	1.02
A_15_P115437	zgc:110366	[NM_001017779]	down	1.23

A_15_P107790	zgc:110687	[NM_001020625]	up	1.33
A_15_P745346	zgc:113142	[NM_001012488]	down	1.42
A_15_P161112	zgc:113625	[NM_001013481]	up	1.22
A_15_P175721	zgc:123068	[NM_001007132]	up	1.21
A_15_P206466	zgc:123294	[NM_001037435]	up	1.16
A_15_P578737	zgc:136383	[NM_001045294]	up	1.53
A_15_P674461	zgc:136410	[NM_001040294]	down	1.14
A_15_P153021	zgc:136909	[NM_001040357]	down	1.44
A_15_P670131	zgc:152791	[NM_001007133]	up	1.61
A_15_P111401	zgc:153154	[NM_001045413]	down	1.10
A_15_P239711	zgc:153258	[NM_001076579]	up	1.43
A_15_P723956	zgc:153911	[NM_001077315]	down	1.48
A_15_P162981	zgc:153911	[NM_001077315]	down	1.45
A_15_P195176	zgc:153955	[NM_001080001]	down	1.27
A_15_P275096	zgc:153955	[NM_001080001]	down	1.06
A_15_P436650	zgc:158281	[NM_001089403]	down	1.23
A_15_P169726	zgc:158404	[NM_001080565]	up	1.29
A_15_P565117	zgc:158419	[NM_001080625]	up	1.02
A_15_P179731	zgc:158494	[NM_198824]	up	1.10
A_15_P564722	zgc:158791	[NM_001080652]	up	1.70
A_15_P271091	zgc:162150	[NM_001110481]	down	1.47
A_15_P409440	zgc:162618	[NM_001089331]	down	1.16
A_15_P730918	zgc:165518	[NM_001100153]	up	1.30
A_15_P695781	zgc:165526	[NM_001099258]	up	1.48
A_15_P745946	zgc:171750	[NM_001109709]	up	1.55
A_15_P491842	zgc:172116	[NM_001159835]	down	1.03
A_15_P706596	zgc:175135	[NM_001114722]	up	1.14
A_15_P634791	zgc:175154	[NM_001114747]	up	1.30
A_15_P627581	zgc:193505	[NM_001128697]	down	1.04
A_15_P290971	zgc:193505	[NM_001128697]	down	1.11
A_15_P489547	zgc:194686	[NM_001130647]	down	1.83
A_15_P631431	zgc:195195	[NM_001136254]	up	1.14
A_15_P558067	zgc:195195	[NM_001136254]	up	1.18

A_15_P520182	zgc:55813	[NM_201514]	down	1.04
A_15_P107639	zgc:55813	[NM_201514]	down	1.21
A_15_P209906	zgc:56706	[NM_200274]	down	1.03
A_15_P530922	zgc:63840	[NM_200626]	up	1.08
A_15_P194351	zgc:64114	[NM_200107]	down	1.29
A_15_P207341	zgc:73230	[NM_213038]	down	1.06
A_15_P438810	zgc:73230	[NM_213038]	down	1.34
A_15_P726416	zgc:77060	[NM_001002218]	down	1.42
A_15_P720746	zgc:77060	[NM_001002218]	down	1.27
A_15_P169431	zgc:77060	[NM_001002218]	down	1.21
A_15_P624031	zgc:85644	[NM_213313]	down	1.26
A_15_P116681	zgc:85866	[NM_001001826]	down	1.04
A_15_P719846	zgc:85866	[NM_001001826]	down	1.20
A_15_P520922	zgc:91910	[EH477938]	up	1.04
A_15_P445650	zgc:92020	[NM_001002647]	down	1.00
A_15_P194471	zgc:92020	[NM_001002647]	down	1.36
A_15_P102446	zgc:92851	[NM_001002493]	down	1.96
A_15_P538682	zp3a.2	[NM_001030120]	up	1.24
A_15_P120078	zymnd12	[NM_001007304]	up	1.46
A_15_P761409			up	1.42
A_15_P408165			up	1.28
A_15_P757861			up	1.29
A_15_P200226		[EG577093]	up	1.58
A_15_P402080			up	1.26
A_15_P725396		[BC121736]	down	1.54
A_15_P513162		[CT691484]	down	1.10
A_15_P180961		[CN328052]	down	1.01
A_15_P289391		[CK396210]	up	1.05
A_15_P675891		[CN512711]	down	1.13
A_15_P107341		[TC393179]	up	1.08
A_15_P198661		[TC414227]	down	1.97
A_15_P181891			up	1.07
A_15_P281206		[XM_003197690]	down	1.54

A_15_P269311	[AL918048]	up	1.56
A_15_P410806	[XM_003200924]	up	1.50
A_15_P204401	[CT611069]	down	1.42
A_15_P498137	[BM035999]	up	1.16
A_15_P144641	[TC366248]	up	1.10
A_15_P188946		up	1.18
A_15_P101201	[XM_686645]	up	1.44
A_15_P265091		down	1.01
A_15_P764051		up	1.15
A_15_P219366	[XM_688443]	down	1.55
A_15_P191876	[TC439458]	down	1.05
A_15_P548202		up	1.07
A_15_P246182	[XM_001919450]	up	1.33
A_15_P518452	[XM_684040]	up	1.02
A_15_P681211	[TC378404]	up	1.03
A_15_P498397	[XM_681237]	up	1.12
A_15_P758191		up	1.01
A_15_P441015	[EE319015]	down	2.32
A_15_P548447	[BC162064]	up	1.50
A_15_P489422	[CN016358]	up	1.50
A_15_P267357	[CK686462]	up	1.12
A_15_P135406		up	1.41
A_15_P685621	[TC420209]	up	1.14
A_15_P241401		up	1.85
A_15_P285996	[CN317944]	up	1.19
A_15_P113950	[TC411220]	up	1.71
A_15_P267166	[XM_001922243]	up	1.12
A_15_P286766	[XM_695099]	up	1.14
A_15_P309971	[TC417813]	down	1.21
A_15_P711016	[CN024216]	up	1.08
A_15_P168471	[TC367136]	up	1.02
A_15_P546147		up	1.24
A_15_P673576	[BC078260]	up	1.60

A_15_P131221	[DQ851810]	up	1.63
A_15_P557582	[EB952200]	down	1.01
A_15_P567777		up	1.31
A_15_P416900	[DN897193]	down	1.77
A_15_P310861	[TC380263]	up	1.10
A_15_P223461		up	1.29
A_15_P119540	[CK015579]	down	1.22
A_15_P265236	[CT640385]	up	1.13
A_15_P138071	[XM_002666233]	down	1.02
A_15_P244356	[TC388477]	down	2.26
A_15_P671861	[TC381056]	down	1.37
A_15_P689631		up	1.20
A_15_P266256	[TC388488]	down	1.88
A_15_P499152	[EH442140]	down	1.20
A_15_P676476	[CV488179]	up	1.06
A_15_P672126	[TC383441]	down	1.14
A_15_P213781	[TC431608]	down	1.68
A_15_P133451	[BC044469]	up	1.16
A_15_P201271	[BC095722]	down	1.60
A_15_P237981		up	1.05
A_15_P177256	[BC115099]	up	1.72
A_15_P290786	[XM_003199061]	down	1.16
A_15_P772801	[CK015579]	down	1.35
A_15_P334704	[EV756923]	down	1.25
A_15_P677791	[TC383083]	up	1.19
A_15_P582192	[TC393521]	down	1.28
A_15_P687508		down	1.04
A_15_P545132	[TC396305]	up	1.21
A_15_P399495	[EV603615]	down	1.70
A_15_P165356	[TC378368]	down	1.13
A_15_P247211	[BC093440]	up	1.54
A_15_P243781	[XR_084445]	down	1.08
A_15_P316291	[XM_001922255]	up	1.06

A_15_P709421		up	1.38
A_15_P111207	[BC085676]	down	1.73
A_15_P740683		up	1.95
A_15_P181793	[TC394273]	up	1.26
A_15_P200036		up	1.07
A_15_P377845		up	1.11
A_15_P116408	[BC110094]	down	1.14
A_15_P237956	[BI888503]	up	1.69
A_15_P460060	[XM_001344894]	up	1.10
A_15_P192081		up	1.36
A_15_P438150	[TC432313]	up	1.24
A_15_P759241		up	1.27
A_15_P759506		up	2.14
A_15_P135931	[EE301006]	up	1.32
A_15_P699656	[XM_002665119]	up	1.88
A_15_P684717	[XM_002666074]	up	1.30
A_15_P190031	[DY548415]	up	1.19
A_15_P727526	[TC390102]	up	1.47
A_15_P285751		down	1.09
A_15_P130766	[DQ017642]	up	1.11
A_15_P113522	[CF348334]	down	1.95
A_15_P435010	[NM_001123373]	up	1.22
A_15_P762036	[TC368069]	up	2.29
A_15_P282106		up	1.35
A_15_P268811	[EB966255]	up	2.28
A_15_P197751	[BC135044]	down	1.51
A_15_P555532	[XM_001923116]	up	1.23
A_15_P726901	[BC159225]	down	1.97
A_15_P761816		up	1.14
A_15_P749901	[TC376951]	up	1.05
A_15_P759001		up	1.18
A_15_P738336	[BC153939]	up	1.01
A_15_P190421	[XM_683088]	up	1.41

A_15_P156806	[TC415982]	up	1.31
A_15_P189571		up	1.10
A_15_P191926	[DQ017628]	up	1.64
A_15_P368435	[TC429594]	down	1.21
A_15_P157636	[XM_002663518]	down	1.54
A_15_P260406		up	1.32
A_15_P671308		up	2.13
A_15_P682312		down	1.35
A_15_P759456		up	1.82
A_15_P112984		down	1.10
A_15_P241556	[TC397077]	down	1.04
A_15_P187211	[BC095167]	down	1.09
A_15_P265881	[EH545089]	down	1.94
A_15_P158376	[TC403649]	down	1.08
A_15_P178951	[BC150453]	down	1.21
A_15_P204321	[DQ017611]	down	1.84
A_15_P740981	[BC128878]	down	1.60
A_15_P597127	[XM_683420]	down	1.10
A_15_P260421		up	1.19
A_15_P158451	[BC076450]	up	1.49
A_15_P393565	[TC377882]	down	1.06
A_15_P100294	[CK239381]	down	1.06
A_15_P554197	[CN322620]	down	1.72
A_15_P404185	[BC155349]	down	1.10
A_15_P197446	[TC369209]	down	1.34
A_15_P594157	[CK018437]	down	1.06
A_15_P551332	[XM_689616]	down	1.14
A_15_P396295	[BC128878]	down	1.97
A_15_P265011	[TC396323]	down	1.04
A_15_P381365	[XM_001339793]	down	1.00
A_15_P239881		down	1.60
A_15_P222371		down	1.33
A_15_P350635	[TC383441]	down	1.81

A_15_P112968	[XM_002666100]	up	1.60
A_15_P378491	[CO918741]	down	1.71
A_15_P266046	[TC387004]	up	1.50

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